Distinctive Characteristics of Crude Interferon from Virus-infected Guinea-pig Embryo Fibroblasts

By TIMOTHY R. WINSHIP, † C. K. Y. FONG AND G. D. HSIUNG*

*Virology Laboratory, Veterans Administration Medical Center, West Haven, Connecticut 06516 and Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06510, U.S.A.

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SUMMARY

Crude interferon preparations from primary guinea-pig embryo cells infected with vesicular stomatitis virus strain T1026R1 were shown to be more sensitive to heat (37 °C), pH 2-0, and SDS than crude mouse interferon. Since the proportion of antiviral activity lost after each treatment was nearly the same, the existence of a single fraction of antiviral activity sensitive to all three treatments was suggested. Support for this possibility was given by the finding that subjecting this guinea-pig interferon to any one of the treatments rendered it insensitive to the effects of the other two.

We have previously shown that guinea-pig embryo fibroblasts (GPE cells) infected with several different viruses were capable of producing more antiviral activity (interferon) than had been previously reported by other laboratories (Kaplan et al., 1962; Friedman et al., 1962; Lackovic et al., 1979; Warfel & Stewart, 1980; Sonnenfeld, 1981; Christofinis, 1980), but that much of this activity was too labile to be detected by conventional means (Winship et al., 1983). It was found that virus type, multiplicity of infection, and cell age are important factors in determining the total yield of these substances (Winship et al., 1983).

In this communication, we describe several basic biological and physical characteristics of crude guinea-pig interferons. These were compared with crude, virus-induced mouse interferons with 'classical' characteristics. It was found that crude guinea-pig interferons were a mixture of conventional interferon and a heat-, pH 2-, and SDS-labile fraction constituting approximately 60% of the antiviral activity.

GPE fibroblasts were prepared from whole guinea-pig foetuses (30 days gestation) as described previously (Hsiung et al., 1976). Primary and passaged GPE cell cultures were grown in culture flasks or roller bottles for interferon induction and for assays as described by Winship et al. (1983). Mouse L-929 fibroblasts and Vero cells were originally obtained from Flow Laboratories. They were grown in Eagle's minimum essential medium (MEM) containing 10% newborn bovine serum (NBS) and maintained in MEM plus NBS, 2% for L-929 and 5% for Vero cells.

Vesicular stomatitis virus (VSV) T1026R1 is a non-ts revertant of a mutant originated by C. P. Stanners (Stanners et al., 1977). Stock VSV T1026R1 was prepared from plaque-purified virus in Vero cells. Wild-type VSV, Indiana strain, was originally obtained from the American Type Culture Collection (no. VR-158). Assays of wild-type VSV could be conducted in Vero, GPE and L-929 cells with nearly equal efficiency.

Guinea-pig interferons were produced in 32 oz culture flasks or roller bottles of confluent GPE cells. For interferon induction, confluent GPE cells were aged for 7 days in growth medium which was maintained at neutral pH by adding a small amount of sodium bicarbonate, without medium change. The aged cells were infected with VSV T1026R1 under conditions previously described (Winship et al., 1983) and fed with MEM containing 0-5% NBS. The infected cultures were incubated at 37 °C for 24 h for interferon production. Following this incubation period, the...
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Fig. 1. Effect of exposure of primary GPE cell (○) and mouse (△) interferons to 37 °C. Antiviral activity remaining in each heated sample is expressed as the percent of antiviral activity exhibited by a freshly thawed (from −70 °C storage) sample of the same guinea-pig or mouse preparation.

interferon-containing medium was rapidly chilled to 4 °C and clarified by centrifugation in a Sorvall GSA rotor for 2 h at 8000 rev/min, and stored frozen at −70 °C until needed.

Mouse L-929 cell interferon was produced and harvested in essentially the same manner following infection by VSV T1026R1. L-929 cells, however, required no ageing period and could be used for interferon production as soon as they reached confluence.

Standard plaque reduction assays of interferon activity were used for all experiments. Interferon activity was expressed in PR50 units (50% plaque reduction titre). GPE fibroblasts were used for assays of guinea-pig interferons. L-929 mouse interferon was equally active on both L-929 cells and GPE fibroblasts. Except where noted, mouse interferon was assayed on GPE monolayers. All interferon assays were performed in duplicate and in the presence of our own 'standard' guinea-pig and mouse interferon preparations, made as described above. For mouse interferon induced by VSV T1026R1 in L cells, 1 PR50 unit was equivalent to about 2 units of the International Reference Standard (G002-904-511) from the National Institutes of Health.

Unless otherwise indicated, the inducing virus in all interferon-containing samples was inactivated prior to assay by exposure to short-wave u.v. light under conditions previously described. A VSV T1026R1 preparation inactivated in this way could not induce interferon, nor interfere with the replication of wild-type VSV (Winship et al., 1983).

The guinea-pig interferon preparation used in all of the following experiments had a titre of 4150 PR50 units/ml, and the crude mouse L-929 interferon preparation used had a titre of 12700 PR50 units/ml on L-929 and 14120 PR50 units/ml on GPE fibroblasts. Both interferon preparations were stored frozen at −70 °C in 50 ml aliquots. Aliquots were never re-frozen following their initial thawing, and the entire batch of each interferon was used within 90 days of its production.

The stability of guinea-pig and mouse interferons at 37 °C was assessed by incubation of 1 ml aliquots of each interferon (at 4 °C) in 5 ml Falcon polypropylene test tubes. After incubation at 4 °C for 1 h to assure temperature uniformity, the first aliquot in each series was immediately frozen at −70 °C and the others were immersed in a 37 °C water-bath. At the time intervals indicated in Fig. 1, individual tubes were removed from the water-bath, rapidly chilled to 4 °C, and then frozen at −70 °C until assayed. At the end of the experiment all the individual aliquots were rapidly thawed and simultaneously assayed for interferon activity on the same lot of GPE cells. Use of a single lot of GPE fibroblasts in the assay of an experiment greatly reduced the degree of error inherent in the plaque reduction method. The results are shown in Fig. 1. The mouse interferon showed essentially no inactivation during the 22 h period of exposure to 37 °C.
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Fig. 2

Fig. 2. Effect of exposure to pH 7.0 (■) or pH 2.0 (■) of interferons from (a) mouse L-929 fibroblasts and (b) primary GPE fibroblasts for the times shown. The antiviral activity remaining in each sample is expressed as the percent of antiviral activity exhibited by a freshly thawed sample of the same guinea-pig or mouse preparation.

Fig. 3

Fig. 3. Response of crude interferon preparations from (a) mouse L-929 cells and (b) primary GPE cells to treatment with 0.01% SDS (■), compared to untreated controls (□), for the times shown. Antiviral activity remaining in each sample is expressed as the percent of antiviral activity of the respective controls.

In sharp contrast, guinea-pig interferon lost approximately 60% of its antiviral activity during the first 3 h of exposure at 37 °C. There was no further significant loss of antiviral activity over the course of the experiment. These data are the results of a single representative experiment; the rapid loss of antiviral activity from guinea-pig interferon preparations treated at 37 °C could be repeated with any other interferon stock prepared from primary GPE cells. GPE and mouse interferon samples held at 4 °C for 22 h showed no loss of antiviral activity.

Aliquots of the same preparations of guinea-pig and mouse interferons were used to determine the effect of exposure to pH 2.0. Twenty-ml samples of u.v.-irradiated mouse and guinea-pig interferons were adjusted to pH 2.0 by the dropwise addition of 5 M-HCl. The pH was continuously monitored with a pH meter. Similar samples of guinea-pig and mouse interferon were adjusted to pH 7.0 with 1 M-HCl and 1 M-NaOH. The samples were held at 4 °C for up to 48 h. At 24 h, 10 ml of mouse and guinea-pig interferons, at pH 2.0 and 7.0 respectively, were removed and the pH 2.0 samples were re-adjusted to neutrality. This process was repeated with the remaining samples at 48 h post-treatment. The total volume of liquid (as HCl or NaOH) added to the 20-ml aliquots did not exceed 1 ml in each case.

After pH adjustment, all samples were re-frozen at -70 °C until assays could be performed. The samples were assayed simultaneously on a single lot of passaged GPE cells. In addition to aliquots that remained at pH 7.0 throughout the experiment, samples of interferon (from the same batches) freshly thawed from -70 °C storage were also assayed to detect possible changes in the antiviral titre during the storage of samples at 4 °C. The results of this experiment are shown in Fig. 2.

The samples of both guinea-pig and mouse interferon adjusted to pH 7.0 showed no significant loss of antiviral activity during the period of exposure to 4 °C for 24 and 48 h. The sample of mouse interferon treated at pH 2.0 also showed no loss of antiviral activity during the 48 h period of exposure. However, the guinea-pig interferon samples treated at pH 2.0 for 24 and 48 h each showed a 60% loss of antiviral activity. Loss of activity was again only partial, a fraction of the guinea-pig antiviral activity being resistant to exposure to pH 2.0.

The response of guinea-pig and mouse interferons to treatment with SDS was measured using
Table 1. Effect of sequential treatments with heat, pH 2 and SDS on antiviral activity in a crude GPE cell-derived interferon preparation

<table>
<thead>
<tr>
<th>Primary treatment of GP IFN with</th>
<th>Untreated activity (PR50 units/ml)</th>
<th>Activity after primary treatment (PR50 units/ml)</th>
<th>Activity after secondary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 °C (3 h)</td>
<td>4120</td>
<td>1440 (35%)</td>
<td>37 °C 1375 (33%)* 1490 (36%)</td>
</tr>
<tr>
<td>pH 2.0 (24 h)</td>
<td>3876</td>
<td>1548 (40%)</td>
<td>pH 2.0 1520 (39%) NA 1452 (37%)</td>
</tr>
<tr>
<td>0·01 % SDS (24 h)</td>
<td>4060</td>
<td>1705 (42%)</td>
<td>0·01 % SDS 1680 (41%) 1420 (35%)</td>
</tr>
</tbody>
</table>

* Percent control antiviral activity remaining.
† NA, Not applicable.

20-ml aliquots of mouse and guinea-pig interferons adjusted to pH 7·0, and to which SDS was added to a final concentration of 0·01% (w/v). Other aliquots were adjusted to pH 7·0 but no SDS was added. The samples were allowed to remain at 4 °C for 24 h, at which time 10 ml of SDS-treated and untreated samples were dialysed overnight at 4 °C against two changes of 1000 ml of MEM containing 0·5% NBS in order to remove residual SDS. The process was repeated with the remaining treated and untreated samples at 48 h post-treatment. The resulting aliquots were frozen at −70 °C until assays could be performed. All samples were assayed simultaneously on a single batch of passaged GPE cells. The results, shown in Fig. 3, indicated that mouse interferons were not sensitive to the presence of 0·01% SDS for a period of up to 48 h at 4 °C. Guinea-pig interferon, however, lost about 55% of its antiviral activity after treatment with 0·01% SDS for 24 h. No further significant decline in antiviral activity was detected after 48 h of exposure to this detergent.

To determine the properties of the antiviral activity remaining in crude guinea-pig interferon preparations treated either with heat, acid pH or SDS, samples treated with each of these agents were subjected to secondary treatment with the remaining two. A 20 ml sample of guinea-pig interferon (from the same batch as that used in the previous experiments) was treated for 24 h at pH 2·0 by the dropwise addition of 5 M-HCl, restored to neutrality with 5 M-NaOH and stored at −70 °C. Another 20 ml sample of the same batch was heated (in individual 1 ml aliquots) for 3 h at 37 °C. The heat-treated interferon was then pooled and stored frozen at −70 °C. A third 20 ml sample was made 0·01% in SDS and allowed to remain at 4 °C (pH 7·0) for 24 h. This was then dialysed overnight against two changes of 1000 ml of MEM plus 0·5% NBS to remove SDS. Each treated sample was assayed for interferon activity to ensure that the predicted partial decline in antiviral effect had occurred. The sample that had been heated was then split into two fractions which were treated individually with acid or SDS for 24 h as previously described. The 20 ml sample that had been treated at pH 2·0 for 24 h was split into two fractions which were individually treated with heat and SDS, again as previously described. Finally, the 20 ml sample that had been treated with SDS was divided into two fractions. One was heated at 37 °C for 20 min and the other exposed to pH 2·0 for 24 h.

Following this series of secondary treatments, all resulting samples were simultaneously assayed on a single lot of passaged GPE cells. Freshly thawed guinea-pig interferon (from the same batch as used previously) was used as an additional control. The results of this experiment, summarized in Table 1, indicate that guinea-pig interferon treated with any one of the three physicochemical treatments described lost about 60% of its antiviral activity, but the approximately 40% remaining after any of these treatments was resistant to further treatment with the other two methods.

Since crude, virus-induced interferon preparations are nearly always subjected to a period of low pH, it is difficult to find evidence for acid-labile interferon activity from cells of other species. Evidence is accumulating, however, that acid-labile interferons are present in the sera of human patients with systemic lupus erythematosus and other diseases of the immune system (Hooks & Detrick-Hooks, 1982; Preble et al., 1982). Such interferons seem to be largely of the alpha variety. We cannot, at this time, rule out the possibility that the unstable antiviral activity...
obtained from virus-infected GPE cells is an atypical alpha-type interferon, although the
properties exhibited are more suggestive of a gamma interferon.

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